

AMENDMENTS TO THE SPECIFICATION

On page 1, please amend lines 7-11 as follows:

The present application is a ~~divisional~~ continuation of U.S. Application Serial No. 09/859,650, filed, May 17, 2001, entitled "Method and Apparatus for Detecting Cancerous Cells Using Molecules that Change Electrophoretic Mobility which is a continuation-in-part application of application Serial No. 09/358,504 filed July 21, 1999, entitled, "Method And Apparatus for Detecting Enzymatic Activity Using Molecules That Change Electrophoretic Mobility", which in turn was a continuation-in-part application of serial No. 09/036,706, filed March 6, 1998, entitled "Fast Controllable Laser Lysis for Cells for Analysis".

At page 26, beginning on line 8 please substitute the following modified paragraph:

The sequence of amino acid residues that comprises this reporter peptide is derived from a region of the PKC protein sequence. The peptide sequence, as found in the larger PKC sequence, Arg-Phe-Ala-Arg-Lys-Gly-Ala-Leu-Arg-Gln-Lys-Asn-Val (SEQ ID NO:1), is known to inhibit the enzyme, but when the underlined alanine residue is substituted with a serine residue, the resulting sequence is known to serve as a relatively specific substrate for PKC. Thus, the sequence of FI-sPKC is FI- Arg-Phe-Ala-Arg-Lys-Gly-Ser-Leu-Arg-Gln-Lys-Asn-Val (SEQ ID NO:2), where "FI" stands for a fluorescein moiety that was covalently attached to the amino terminal end of the peptide following standard peptide synthesis. The underlined serine residue, Ser, is the phosphorylation site. Resin-bound, nonfluorescent sPKC peptide was obtained from the Stanford PAN facility and generated by conventional synthetic methods. The peptide with fluorescein covalently bound to the amino terminus was prepared by incubating 5-carboxyfluorescein succinimidyl ester (100-200 mg/ml, Molecular Probes, Eugene, OR) with resin-bound nonfluorescent peptide (30 mg/ml peptide), 1-hydroxybenzotriazole (57 mg/ml), and N,N diisopropylethylamine (55 mg/ml) in dimethylformamide for 12 hours on a rotating mixer. The peptide was washed with dimethylformamide and ethyl acetate, and then cleaved from the resin by incubation for two hours with a trifluoroacetic acid (TFA)/free-radical scavenger mix (trifluoroacetic acid (84%, v/v), phenol (0.2%, wt/v),

thioanisol (5%, v/v), ethanediol (2.5%, v/v), water (8.5%, v/v)), previously sparged with argon or nitrogen. The peptide was separated from the resin by passage through a sintered glass filter. The peptide was then precipitated by addition of ice cold ether, dissolved in acetic acid (5%), and subsequently lyophilized. The peptide was purified by reverse phase high pressure liquid chromatography (HPLC) on a semipreparative C18 column (Alltech, Deerfield, IL) with a gradient of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile. The concentration of the fluorescein-labeled peptide, FI-sPKC dissolved in buffer A (135 mM NaCl, 5 mM KCl, 10 mM HEPES, 2 mM MgCl₂, and 2 mM CaCl₂ adjusted to pH 7.4 with NaOH), was determined by comparing its fluorescence to that of standards of hydrolyzed carboxyfluorescein succinimidyl ester. FI-sPKC purity was assessed by reverse phase high pressure liquid chromatography on an analytic C18 column (Alltech) and by capillary electrophoresis. The peptide was stored in 2 aliquots at -70° C.

At page 49, beginning at line 17 please substitute the following modified paragraph:

Fig. 7 is a graph of phosphorylation as a function of time showing the separation of three different specific kinase reporter substrates from a sample of the contents of a *Xenopus laevis* oocyte **46'** that had been previously microinjected to contain ~1 μ M FI-sPKC, ~330 nM FI-sPKA, and ~10 nM FI-scdc2K. FI-sPKA, a specific reporter for protein kinase A (PKA) activity has the sequence FI-Lys-Arg-Arg-Glu-Ile-Leu-Ser-Arg-Arg-Pro-Ser-Tyr-Arg (SEQ ID NO:3), and was derived from the CREB protein. FI-scdc2K, a specific reporter for cdc2 kinase (originally identified genetically as cell division cycle mutant 2) has the sequence FI-Gly-Gly-Gly-Arg-Ser-Pro-Gly-Arg-Arg-Arg-Arg-Lys (SEQ ID NO:4), and comprises a consensus phosphorylation site derived from several proteins. The underlined serine residues are the sites of phosphorylation. The peptides were synthesized and labeled with fluorescein as described for FI-sPKC, except that FI-scdc2K was labeled with the mixed 5- and 6-isomers of carboxyfluorescein succinimidyl ester (100-200 mg/ml, Molecular Probes, Eugene, OR); thus, FI-scdc2k consisted of two isomeric forms. A peak **162** and a peak **172** were identified by their migration times as observed when injected into oocytes **46'** singly (not

shown). The first doublet, peaks **162** and **164**, corresponds to two isomers of either phosphorylated or nonphosphorylated FI-scdc2K. The second doublet, peaks **166** and **168**, corresponds to two isomers of the other form of FI-scdc2K. One peak **170** represents nonphosphorylated FI-sPKC, and one peak **172** represents nonphosphorylated FI-sPKA.

At page 59, beginning on line 17 of page 59 please substitute the following modified paragraph:

Fig. 7 is an electrophoretogram showing the separation of three different specific kinase reporter substrates from a sample of the contents of a *Xenopus laevis* oocyte that had been previously microinjected. In the measurement illustrated in Fig. 7, an individual oocyte **46'** was microinjected with 50 nl of a mixture of reporter substrate peptides. The mixture was comprised of 20 μ M FI-sPKC, 7 μ M FI-sPKA, and 500 nM FI-scdc2K in buffer B. FI-sPKA, a specific reporter for protein kinase A (PKA) activity has the sequence FI-Lys-Arg-Arg-Glu-Ile-Leu-Ser-Arg-Arg-Pro-Ser-Tyr-Arg (SEQ ID NO:3), and was derived from the CREB protein. FI-scdc2K, a specific reporter for cdc2 kinase (originally identified genetically as cell division cycle mutant 2) has the sequence FI-Gly-Gly-Gly-Arg-Ser-Pro-Gly-Arg-Arg-Arg-Arg-Lys (SEQ ID NO:4), and comprises a consensus phosphorylation site derived from several proteins. The underlined serine residues are the sites of phosphorylation. The peptides were synthesized and labeled with fluorescein as described for FI-sPKC, except that FI-scdc2K was labeled with the mixed 5- and 6-isomers of carboxyfluorescein succinimidyl ester (100-200 mg/ml, Molecular Probes, Eugene, OR); thus, FI-scdc2k consisted of two isomeric forms. Each oocyte **46'** was incubated in buffer C for 30 minutes to allow diffusion of the reporter substrates. The estimated final intracellular concentrations of reporter substrates were ~ 1 μ M FI-sPKC, ~330 nM FI-sPKA, and ~10 nM FI-scdc2K. Separation was achieved with PERT CE sampling followed by MEKC with buffer D through a capillary with an inner coating of PEI. In the measurement, the reservoir that contained the oocyte **46'** was filled with buffer C, while the capillary **22**, the second reservoir **134**, and the outlet reservoir **34** contained buffer D.